Memorial Sloan Kettering Cancer Center

# FACSAria™ Unclog Procedure

Clogged Nozzle

Clear Nozzle

Flow Cytometry Core Facility

Ver. #1.0
2020-04-15

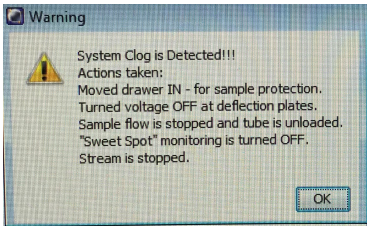
## Workflow Overview

Unclog Procedure for FACSAria™ Sorters

Clog detection & Stream stoppage ▶ Clean sorting chamber ▶ Check & clean the nozzle ▶ Re-start the stream and QC ▶ Continue Sorting

## 1. Clog detection & Stream stoppage

A clog occurs when there is an obstruction of the nozzle. If the nozzle is obstructed, the stream may be blocked entirely, or in the case of a mild obstruction the stream may be deflected or droplets may be irregular in shape. Sweet spot monitors drop formation, and if there are no drops detected due to the clog, it will stop the stream and notify of a potential clog.

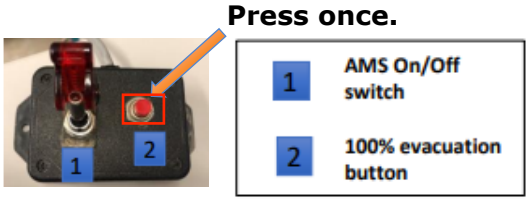



**Note:** Even though clog is often automatically detected by Diva, circumstances occur where the stream is simply deflected and Sweet Spot will not stop the stream. In these cases, there is a high likelihood of contaminating the sorted samples or of wetting the high voltage plates and causing severe damage to the instrument. It is highly encouraged for the operator to be present at all times during the sort to monitor the stability of stream and threshold rate so that the stream can be turned off manually, in case of a slight clog.

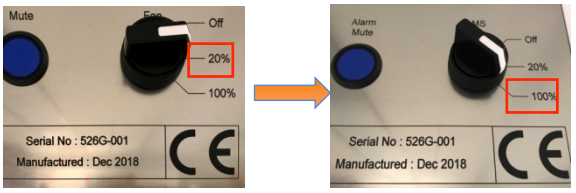
Also note that Sweet Spot may also turn of the stream and notify of a potential clog in situations other than a clog (pressure issues; air in the fluidic lines, etc)

1. When clog occurs during the sorting process, stop the sort and turn of the stream immediately. Ask anyone in the area surrounding the sorter to evacuate.

2. Increase AMS from 20% up to 100% and wait **1 min** to evacuate aerosolized particles generated during clogging from the sort chamber. Turn it back down to 20% before opening the sorting chamber.




**AMS Control for Aria 2/4**




**AMS on BSC for Aria7**

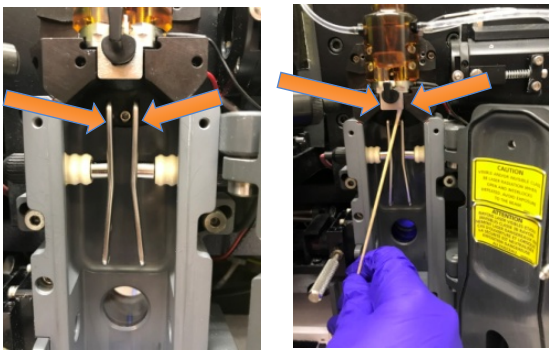
3. Open the sorting chamber and cap the sample tube and the collection tubes and leave them in a safe place.

## 2. Clean the sorting chamber

1. Ensure the deflection plate Voltage is off before opening the sorting chamber.

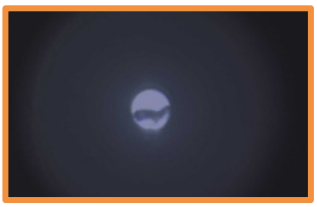
   **Note:** Check that the red warning light will be illuminated if the plates are charged.

2. The sort chamber surface and plates can be cleaned with Kimwipe moistened with 70% EtOH. Especially pay attention to both sides of deflection plates and the stream camera.



## 3. Check and clean the nozzle

1. Remove the nozzle and inspect it under microscope. If any obstruction is seen, sonicate it for one minute in a 15 mL conical tube with $H_2O$. Check the nozzle after sonication to make sure it is clean and no obstruction is seen. If still clogged, repeat sonication for another minute until clear.



**Obstruction in Nozzle Orifice**

**Clear Nozzle Orifice**

2. Insert the clean nozzle back into the flow cell.

## 4. Re-start the stream and QC

1. Turn on the stream by clicking the red "X" at the top of the stream window and verify that the drop 1 and gap are unchanged from original setup when QC was carried out.
2. Open Accudrop Experiment in QC Folder.
3. Re-calculate Drop-Charge Delay with Accudrop beads.

   **Note:** Check ▶***FACSAria™ Drop-Charge Delay SOP***.
4. Re-do the side stream testing for the appropriate collection devices (including plate calibration.

## 5. Continue Sorting

1. Clean the sort collection tube holder and sort chamber with 70% ethanol or 10% bleach.
2. Load new collection tube(s) or plate.
3. Filter the sample with appropriate size cell strainer before acquisition and resumption of sorting.

**Note:** If there are any concerns about impurity of the sort due to deflected streams with the clog, a post sort purity check is recommended. See ▶***Post sort purity check SOP***.